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Preliminary Report on the Effects of Totigestational Exposure to Ethchlorvynol

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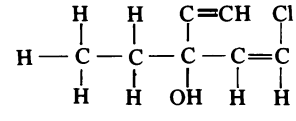
Ethchlorvynol, a sedative-hypnotic drug used clinically since 1955, has recently been the subject of renewed interest primarily because of its chemical relationship to vinyl chloride. In our totigestational studies, sperm-positive female rats were given a daily dose of ethchlorvynol dissolved in olive oil for 21 consecutive days. The dams were allowed to deliver and their offspring were observed for alterations in development by monitoring a number of gross behavioral, histological and biochemical parameters at newborn, weanling, puberty, adult and geriatric stages. Gross development appeared normal at time of weaning; however, offspring of treated dams showed increased behavioral activity in addition to alterations in a number of clinical chemistry parameters. The dose-response seen with most of the parameters suggests that the changes are drug related. However, the clinical pathological significance has not been ascertained.

There are numbers of studies tying vinyl chloride exposure to angiosarcoma of the liver (*1, 2*), other hepatic diseases (*3, 4*), chromosome aberrations (*5*), alterations in spleen (*6*), and pulmonary function (*7*), in addition to other pathological processes. All of these observations place an urgency in studying the mechanisms and far reaching consequences of this and related compounds.

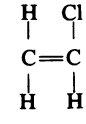
Ethchlorvynol is a chemical somewhat related to vinyl chloride which has the capacity for altering the function of many physiological processes, most of which have been related to its CNS depressant and skeletal muscle-relaxant effects. It was introduced in 1955 as a clinically useful sedative-hypnotic under the trade name Placidyl and has competed quite effectively with the barbituric acid derivatives in some areas. The physiological and biochemical mechanisms by which these effects are elicited have not been well defined. Only as recently as the early 70's has there been any significant attempt to understand it's disposition in either animals or man.

Cummins et al. (*8*), in a clinical study showed that the half-life of absorption for ethchlorvynol is approximately 0.3 hr. The decline in serum levels of the drug was found to be biphasic, suggesting that it is stored, most likely in adipose tissue compartments. On assuming a two-compartment model, the rate constant of elimination was calculated to be 0.13 hr. Cummins also found that ethchlorvynol was readily metabolized in the liver with only 0.025% of the dose excreted unchanged or as the glucuronide.

Another disposition study, perhaps a little more applicable to our work, was reported by Hume et al. (*9*). These investigators showed that an oral dose of ethchlorvynol given to pregnant dogs is rapidly absorbed and readily distributed to the fetal circulation in concentrations comparable to that found in the maternal blood. Little if any delay was seen in the drug reaching the fetal compartment.



Ethchlorvynol



Vinyl chloride

The various metabolites of ethchlorvynol have not been identified; however from the chemical structure, one might think conjugation as the glucuronide would be very likely. However, this metabolite has been looked for but has not been found. Other possible metabolites, such as other conjugates, a cy-

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Table 1. Effect of totigestational exposure to ethchlorvynol on perinatal development.[a]

	Control	Ethchlorvynol 20 mg/kg	Ethchlorvynol 80 mg/kg
Maternal weight, g			
Day 0	156 ± 2	154 ± 2	154 ± 1
Day 7	161 ± 2	162 ± 2	160 ± 2
Day 14	182 ± 3	184 ± 3	178 ± 3
Day 21	226 ± 3	230 ± 2	224 ± 3
Day 22	171 ± 3	175 ± 3	173 ± 3
Sex ratio (M/F)	67/67	59/80	50/46
Litter size	9.6 ± 0.4	9.9 ± 0.5	9.2 ± 0.5
Weight of pups, g			
Birth	5.2 ± 0.1	5.2 ± 0.07	5.3 ± 0.1
1 Week	11.0 ± 0.2	11.9 ± 0.2	11.2 ± 0.2
2 Week	20.1 ± 0.4	21.1 ± 0.3	20.6 ± 0.4
3 Week	29.2 ± 0.6	31.2 ± 0.5	30.2 ± 0.7
4 Week	56.0 ± 1	59.4 ± 0.9	58.7 ± 0.9

[a]Values are means ± S.E. for 10 to 14 litters.

clized five-membered ring, or products of hydroxylation, reduction of unsaturated bonds, splitting off of HCl or vinyl chloride, etc. need to be considered. However, none of these metabolites has been identified.

Our studies on the transplacental pathology of ethchlorvynol were initiated because of: The lack of information of transplacental effects on development and the potential for long term pathology in the offspring, the clinical usage in humans, the absence of metabolite information associated with the possibility of the vinyl chloride moiety of ethchlorvynol reacting in a similar way to vinyl chloride in the intact molecule or as a metabolite, and the occurrence of other unexplained complications associated with ethchlorvynol therapy.

Our studies, which are just beginning, are designed to provide information on the effect of ethchlorvynol on offspring of females subjected to totigestational exposure. Sperm-positive female rats were divided into three groups, and each group was given a daily oral dose of olive oil (control) or 20 or 80 mg/kg ethchlorvynol dissolved in olive oil for 21 days. The dams were allowed to deliver and raise their offspring. The offspring were observed for gross abnormalities and subjected to a number of behavioral, developmental, histological, and biochemical tests at various stages of development. The specific parameters monitored are shown in Tables 1–7. The data presented in the tables are for the most part as the means ± S.E. Control–treated comparisons were made using Student's *t;* the level of significance is $p < 0.05$.

Table 1 presents maternal and newborn statistics. No significant differences were seen between control and treated groups, however treated animals tend to be slightly heavier by 3 to 4 weeks.

Table 2 shows the age of the animals at time of eye opening and onset of puberty in females as determined by vaginal opening. By day 56 all groups showed 100% vaginal opening.

Table 2. Effect of totigestational exposure to ethchlorvynol on eye opening and vaginal opening.

	Proportion of total offspring responding		
	Control	Ethchlorvynol 20 mg/kg	Ethchlorvynol 80 mg/kg
Eye opening[a]			
15 days	0	0	0
16 days	12	8	13
17 days	35	39	36
18 days	75	79	73
19 days	100	100	100
Vaginal opening[a]			
31 days	0	0	0
39 days	29	33	39
46 days	76	67	82
49 days	79	72	89
53 days	87	93	96

[a]Days of postnatal life.

Table 3 shows no difference in control versus treated groups in the three physiological parameters studied. However, treatment causes a trend toward decreased time for righting, suggesting increased activity in the offspring.

The results of behavioral tests are seen in Table 4, which shows a dose-response increased exploratory activity in both male and female weanling animals. Treated male animals tend to require less time to run the food maze with fewer mistakes than do the controls; however a dose response was not seen. Treated females were practically identical to the control females.

Clinical chemistry and hematological observations in weanling animals are presented in Tables 5 and 6. Several parameters—blood glucose, chloride, albumin, total bilirubin, SGOT, acid–base balance, and creatine phosphokinase—were all

Table 3. Effect of totigestational exposure to ethchlorvynol on righting reflex, body temperature regulation, and muscle strength.

	Control	Ethchlorvynol 20 mg/kg	Ethchlorvynol 80 mg/kg
Righting reflex time, sec[a]	1.6 ± 0.2	1.2 ± 0.2	1.2 ± 0.2
Temperature regulation			
7 days, sec[b]	146 ± 7	131 ± 5	150 ± 14
14 days, °C/5 min[c]	0.96 ± 0.2	1.22 ± 0.2	0.86 ± 0.2
Inclined screen, sec[d]			
7 days	61.2 ± 6	59.0 ± 5	63.0 ± 10
14 days	180 ± 18	168 ± 24	216 ± 24

[a]Time for the animals to turn from back to feet; values are means ± S.E.
[b]Length of time needed to lose 1°C of body temperature.
[c]Body temperature change in 5 min.
[d]Length of time animals would adhere to a screen inclined at 45° angle (7 days) or at a 75° angle (14 days).

Table 4. Effect of totigestational exposure to ethchlorvynol on behavior[a]

	Control		Ethchlorvynol 20 mg/kg		Ethchlorvynol 80 mg/kg	
	Males	Females	Males	Females	Males	Females
Openfield test[b]	33 ± 2	38 ± 4	41 ± 3	44 ± 2	44 ± 3	47 ± 2
Food maze, 24 hr[c]						
Time, sec	137 ± 43	58 ± 8	63 ± 13	68 ± 28	105 ± 28	57 ± 8
Mistakes	12 ± 3.0	7 ± 1.3	4.6 ± 1	4.2 ± 1	7.9 ± 2	6 ± 1
Food maze, 48 hr[c]						
Time, sec	41 ± 22	28 ± 7	23 ± 7	38 ± 5	23 ± 5	33 ± 10
Mistakes	4.6 ± 1.3	3.5 ± 0.1	2.5 ± 8	4.1 ± 1	3 ± 0.2	4.8 ± 1.4

[a]Values are means ± S.E. for 8 animals at 4 to 5 weeks of age.
[b]The number of squares visited during 60 sec in the testing device.
[c]The length of time to run the maze following 24 and 48 hr food deprivation and the number of mistakes made during the run.

Table 5. Effect of totigestational exposure to ethchlorvynol on clinical chemistry.[a]

	Control	Ethchlorvynol 20 mg/kg	Ethchlorvynol 80 mg/kg
Glucose, mg/dl	151 ± 1	150 ± 2.6	172 ± 6[b]
BUN, mg/dl	16 ± 1	14 ± 0.6	15 ± 1.5
Creatinine, mg/dl	0.5 ± 0.02	0.45 ± 0.02	0.425 ± 0.05
Sodium, meq/l.	142 ± 1	141 ± 0.2	140 ± 0.25
Potassium, meq/l.	5.3 ± 0.2	5.3 ± 0.3	5.2 ± 0.2
Chloride, meq/l.	105 ± 0.5	106 ± 0.2	108 ± 0.1[b]
Carbon dioxide, meq/l.	28 ± 1	26 ± 1.2	26 ± 1
Uric acid, mg/dl	2.6 ± 0.2	2.5 ± 0.2	2.0 ± 0.1
Cholesterol, mg/dl	101 ± 3	96 ± 1.8	93 ± 2.5
Triglyceride, mg/dl	112 ± 8	98 ± 2.2	78 ± 12
Albumin, g/dl	4.8 ± 0.05	4.7 ± 0.06	4.5 ± 0.05[b]
Total protein, g/dl	4.8 ± 0.05	4.8 ± 0.08	4.6 ± 0.05
Calcium, mg/dl	10.8 ± 2	10.4 ± 0.03	10.4 ± 0.1
Phosphorus, mg/dl	9.2 ± 0.2	8.9 ± 0.16	8.6 ± 0.1
Total bilirubin, mg/dl	0.08 ± 0.02	0.06 ± 0.02	0.01 ± 0[b]
SGOT u/l	156 ± 10	129 ± 5	117 ± 5[b]
SGPT u/l	56 ± 4	52 ± 4	47 ± 1.5
(Na-[Cl + CO_2]) (Balance)	9.5 ± 0.3	8.8 ± 0.4	6.0 ± 1[b]
Alkaline phosphatase, u/l	902 ± 30	835 ± 80	848 ± 88
Creatine phosphokinase, ul	933 ± 103	611 ± 55	559 ± 56[b]

[a]Values are mean ± S.E. for 10 weanling animals.
[b]Statistically different from control.

Table 6. Effect of totigestation exposure to ethchlorvynol on blood morphology[a]

	Control	Ethchlorvynol 20 mg/kg	Ethchlorvynol 80 mg/kg
MCHC, %	32.4 ± 0.4	31.5 ± 0.8	32.5 ± 0.5
MCH, pg	20.8 ± 0.3	20.8 ± 0.7	20.9 ± 0.4
MCV, μm^3	64.8 ± 1.2	66.2 ± 0.8	65.0 ± 0.8
PCV, %	35.1 ± 0.5	36.0 ± 0.4	34.8 ± 0.9
Hb, g/dl	11.4 ± 0.16	11.3 ± 0.3	11.3 ± 0.4
RBC × 10^6	5.46 ± 0.06	4.47 ± 1.1	5.42 ± 0.11
WBC × 10^3	2.8 ± 0.1	2.7 ± 0.16	3.2 ± 0.3
Differential			
Seg, %	8.6 ± 0.6	6.4 ± 1.2	6.2 ± 1
Band, %	5.2 ± 2	4.0 ± 1.2	3.8 ± 1.5
Lymph, %	82 ± 2.4	83 ± 2.8	87 ± 1.3
Mono, %	2.8 ± 1	5.2 ± 1.6	3.5 ± 0.5
Eosin %	1.2 ± 0.6	1.2 ± 0.6	1.0 ± 0.6

[a]Values are the means ± S.E. for 10 weanling animals.

statistically different from those at the controls. A number of the parameters showed a dose-response relationship which supports the idea of a drug-related effect. However, the biological significance is not obvious at this point in our experiments. A repetition of these results in another group of weanling animals or in animals of puberty age would add to the significance of these results.

No changes were seen in the hematological parameters. In animals autopsied at 5 weeks of age the 80 mg/kg treatment group was significantly heavier than the control, while the 20 mg/kg group was intermediate (Table 7). It is interesting to note that the mean weight of the brain, pituitary, thyroid, liver, epididymis, and uterus was smaller in the 80 mg/kg-treated animals than in the controls, in spite of the increased body weight.

As pointed out earlier, these data are preliminary and need to be repeated in another group of weanling animals or supported by data from puberty-aged animals. Data just obtained in puberty animals tend to confirm the idea that totigestational treatment with ethchlorvynol causes increased activity in the offspring and further suggest that the increased activity may be of a somewhat random nature.

Table 7. Effect of totigestational exposure to ethchlorvynol on organ weight.[a]

Organ	Control	Ethchlorvynol 20 mg/kg	Ethchlorvynol 80 mg/kg
Whole body, g	56.2 ± 1.1	60.7 ± 1.8	61.6 ± 1.3[b]
Brain, g	1.56 ± 0.06	1.58 ± 0.04	1.50 ± 0.02
Pituitary, mg	4.1 ± 0.6	3.9 ± 0.4	2.8 ± 0.1
Thyroid, mg	14.6 ± 1.7	12.9 ± 1.0	13 ± 1.1
Lung (R), g	0.381 ± 0.03	0.386 ± 0.01	0.363 ± 0.017
Lung (L), g	0.208 ± 0.01	0.222 ± 0.01	0.225 ± 0.007
Heart, g	0.229 ± 0.01	0.241 ± 0.01	0.240 ± 0.007
Thymus, mg	206 ± 13	213 ± 11	220 ± 9
Kidney (R), g	0.301 ± 0.02	0.303 ± 0.01	0.299 ± 0.013
Kidney (L), g	0.288 ± 0.02	0.293 ± 0.01	0.292 ± 0.015
Adrenal (R), mg	7.9 ± 0.4	9.8 ± 1	7.9 ± 0.4
Adrenal (L), mg	9.2 ± 0.7	11.0 ± 0.5	9.3 ± 0.5
Liver, g	2.55 ± 0.1	2.57 ± 0.1	2.50 ± 0.13
Spleen, g	0.181 ± 0.01	0.188 ± 0.01	0.192 ± 0.01
Pancreas, g	0.245 ± 0.01	0.226 ± 0.01	0.253 ± 0.02
Prostate, mg	73 ± 8	82 ± 8	86 ± 3
Testicles, g	0.337 ± 0.015	0.347 ± 0.01	0.378 ± 0.03
Epididymis, mg	61 ± 10	45 ± 5	46 ± 4
Ovaries, mg	26 ± 2	26 ± 3	27 ± 5
Uterus, mg	39 ± 12	38 ± 5	29 ± 7

[a]Values are means ± S.E. Animals were 5 weeks old.
[b]Statistically different from control.

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